Supplementary Figure 1A. Consort flowchart.

Supplementary Figure 1B. Study timeline.

Supplementary Figure 2. Formation dynamics of Dz13 lipoplex by flow cytometry.

(A, B) Comparison of FAM-labelled Dz13 10μg lipoplex and unlabelled Dz13 on FSC/SSC plot and FSC/FAM plot, respectively. The Dz13 lipoplex appeared as a single population on the forward scatter/side scatter (FSC/SSC) plot (A), where generally, FSC correlates with particle size and SSC depends on inner complexity. The FAM-labelled Dz13 lipoplex population

overlapped with unlabelled Dz13 on the FCS/SSC plot, but with greater fluorescence intensity in the FAM channel (B). This confirmed that the lipoplex containing Dz13 was indeed detected by flow cytometry.

(C, D, E) Lipoplex stability over time for the 10, 30 and 100µg formulation, respectively. The formation dynamics of the lipoplex was examined by comparing samples immediately after mixing with samples various times post-mixing up to 4 h. The FSC/SSC plot showed that the sample shifted from the initial dispersed population of low FSC and SSC intensities at 0 min to a single, high-FSC and SSC intensity population at 5 min, suggesting the lipoplex were able to stabilize within a few minutes. The sample remained largely the same on the FSC/SSC plot between 5 and 120 min. At 4 h, a dispersed population with decreased FSC and SSC intensity was observed. This suggested that the lipoplex was morphologically stable for at least 120 min. Similar stability was observed with all three dose formulations (10, 30 and 100µg) (C-E)**.**

(F) Comparison of lipoplex of 10, 30 and 100µg formulation on FSC/SSC plot. The lipoplex that formed with all three Dz13 doses used in this trial (10, 30 & 100µg) after 20 min, overlapped on the FCS/CCS plot, indicating a high degree of similarity in size and morphology irrespective of DNAzyme dose.

(G) Effect of buffer ionic strength on the stability of 10µg lipoplex formulation. The 10µg formulation lipoplex were prepared in water, DMEM and 10x PBS buffer (1.5 M NaCl, 100 mM phosphate, pH 7.4), respectively, and after 20 min assessments made by flow cytometer. The lipoplex prepared in either water or 10x PBS showed a largely dispersed population compared to that in DMEM, indicating the ionic strength of the buffer plays crucial rule in lipoplex stabilization.

Supplementary Figure 3. Microscopic characterization of Dz13 lipoplex.

(A) Scanning electron micrograph (SEM) of representative dried DNAzyme-lipoplex. Scale bars are 1µm.

(B) Atomic force microscopy (AFM) of dried DNAzyme-lipoplex. ***Upper panel*** represents the amplitude (surface characteristics) and ***lower panel*** represents the corresponding height of the sample. Lipoplexes are depicted with an arrow.

The lipoplex was characterized using SEM and AFM. The lipoplex samples were prepared according to the 20 min protocol. The samples were drop cast onto substrates, followed by

rinsing and drying. Representative images of SEM and AFM indicate the presence of mostly large aggregates and high polydispersity, while the large population of primary particles is 100-200 nm in size.

Supplementary Figure 4. Study drug stability. Variation in mass error from the theoretical mass recorded each month for Dz13 (**A**), DOTAP (**B**) and DOPE (**C**). Root mean square error for samples were 196, 33 and 46 ppm. Expected errors for instruments are < ± 400ppm for oligonucleotide and < ± 50ppm for lipid sample measurements. Figure indicates individual injection times for the 3 dose groups (10, 30, 100µg shown as green, blue and red arrows respectively), and the structures of Dz13, DOTAP and DOPE.

APPENDIX

Phase I First-in-Human Clinical Trial of Dz13 DNAzyme Targeting c-*jun* in Patients with Nodular Basal Cell Carcinoma (DISCOVER Trial)

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Preparation and administration of study drug

Each formulation was freshly prepared from frozen stocks (-20^{o}C) under sterile conditions immediately prior to injection in a certified Biohazard Class II hood at room temperature. The DOTAP/DOPE lipid mixture was added to the Dz13/DMEM solution in sterile Eppendorf (polypropylene) tubes and mixed for 15 min at 22^{o}C (**Supplementary Table 2**). This drug formulation procedure was strictly adhered to under independently monitored standard operating practices (Datapharm Australia Pty Ltd) to ensure that a fresh preparation of the test item was administered each time. Of the 100µl final solution, 50µl was drawn into a 31-gauge subcutaneous syringe (BD, North Ryde, NSW, Australia) for patient administration. The remainder was stored at -20^{o}C as a retention sample.

The injection into the substance of the target BCC used a technique analogous to routine local anesthetic administration. The needle was advanced at an angle to the base of the body of the tumor avoiding the apex of the tumor from where the diagnostic biopsy had been taken. The 50µl study drug was injected slowly as the needle was drawn back. A slight transient blanching of the tumor provided a visual confirmation that the study drug was occupying the body of the tumor. The volume delivered intratumorally was assessed by measuring any residual volume in the syringe or estimating any post-injection leakage of study drug from the tumor surface.

Stability testing

To ensure stability of the DNAzyme and lipid components of the study, spectrophotometric analysis was performed on aliquots of frozen stocks at -20^{o}C before, during and after the conclusion of the clinical trial. This was performed on a monthly basis at the Bioanalytical Mass Spectrometry Facility of the University of New South Wales. The patients were not injected at the same time but at staggered times throughout the course of the trial after stability was confirmed by this analysis. The mixed lipid carriers (DOTAP/DOPE) were analyzed by static nanospray analysis using a QStar Pulsar I mass spectrometer (Applied Biosystems, CA, USA), whereas Dz13 was analyzed using the 1100MSD mass spectrometer (HP, CA, USA). There was no change in Dz13, DOTAP or DOPE stability (**Supplementary Figure 2**).

Clinical assessments

Assessments were performed at each visit as outlined in **Supplementary Table 8**. Routine laboratory investigations on blood samples were performed by the Sydney South West Area Pathology Service with the reference ranges shown in **Supplementary Table 9.**

Demographics and baseline characteristics. Information such as age, gender, race, skin type, genetic predisposition to skin cancer, alcohol/drug use, amount of sun exposure and method of contraception was determined at the screening visit. Previous and current medical and surgical histories including concomitant medications were also recorded. New or changes to medications were recorded at each visit.

Physical examination. Vital signs (height, weight, standing and supine blood pressure, resting pulse rate, temperature and respiratory rate) and systems review (general appearance, skin, cardiovascular, respiratory, gastrointestinal, ear/nose/throat, vision, neurology, musculoskeletal and lymph node examination) were performed at each visit.

Hematology. Blood samples were collected for hemoglobin, red blood cell count, packed cell volume, mean cell volume, mean cell hemoglobin concentration, white blood cells with differential counts and platelet count.

Biochemistry. Blood samples were also tested for electrolytes (sodium, potassium, chloride, urea, creatinine), estimated glomerular filtration rate, liver function tests (alanine transaminase, aspartate transaminase, gamma glutamyltransferase, alkaline phosphatase, bilirubin, protein and albumin), lactate dehydrogenase, random blood glucose, and lipid profile (total cholesterol and triglyceride).

Coagulation studies. Activated partial thromboplastin time (APTT), prothrombin time (PT) and international normalized ratio (INR) were determined for each sample.

Immunology. Immunoglobulins G, A, M, and E, as well as antinuclear antibody assessments were performed.

Urinalysis. Urine samples were tested for glucose, ketone, pH, specific gravity, blood, nitrite and white cells using the bedside dipstick test (BD, North Ryde, NSW, Australia). Samples were also analyzed for cell counts, and for the presence of any casts, crystals or bacteria.

Electrocardiogram (ECG). A 12-lead ECG was performed for assessment of the rhythm, rate and QT/QTc intervals, and other cardiac abnormalities at each visit.

Micrometry, dermoscopy and digital photography. The diameter of the target lesion was measured in two perpendicular directions, and the dermoscopic features of the tumor were assessed at 10x magnification. Clinical and dermoscopic photographs were archived.

Punch biopsy. A 2mm biopsy was performed using a disposable 2mm punch blade (Kai Medical, Oyana, Japan) from the center of the target lesion under local anesthetic at the screening visit. Samples were fixed in 10% formalin and embedded in paraffin for preparation of routine H&E stained sections that were examined by pathologists at RPAH.

Excision of target lesion. The target BCC was surgically removed in accordance with standard clinical practice 14 day post-injection, fixed in 10% formalin and embedded in paraffin for examination of H&E sections by RPAH pathologists.

Safety and tolerability

Adverse events. Any clinically significant changes throughout the study were noted, and all toxicities or adverse events arising during study participation were graded according to the Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0 (Cancer Therapy Evaluation Program, National Cancer Institute, NIH, US).

Data Safety Monitoring Board (DSMB) and Area Human Research Ethics Committee (HREC) Review. A panel of experts was established in the form of a DSMB whose role it was to review all the safety data in relation to the sentinel patient and all patients at the end of each cohort and to determine whether it was appropriate to proceed to the next dose level. The DSMB adhered to pre-specified remit and schedule throughout the trial. Two of the three members of the DSMB (RD, NKH), including the chair (NKH), were completely independent in that they had no role in study design, data analysis, data collection or decision to publish. The third member (HTA) contributed to study design prior to the commencement of the trial and is a contributing author of this manuscript. Dose limiting toxicity criteria are specified in **Supplementary Table 10.** Furthermore, a detailed report of participant progress was reviewed by the Area HREC upon completion of each subject participation.

Pharmacokinetic assessments

Collection of samples. It is a phase I requirement that systemic pharmacokinetics assessment of the study drug be conducted. This is particularly important for a first-in-human, first-in-class trial. Plasma samples were collected for pharmacokinetics analysis to determine any systemic exposure from the intratumoural injection even though it was considered unlikely prior to the commencement of the study of systemic Dz13 exposure following local administration of such a relatively small quantity. Five ml of whole blood was collected into potassium EDTA vacutainers at each sampling time point (0, 0·5, 1, 2, 4, 8, 12 and 24 hours, and 7, 14 and 28 days after Dz13 intratumoral injection). The samples were centrifuged at 1200-1500g for 15 min, and 1 ml plasma was transferred to a cryovial containing Herring sperm DNA (Sigma-Aldrich, Castle Hill, NSW, Australia) and stored at -80^{o}C prior to transport to Charles River Laboratories (Edinburgh, UK) for independent analysis.

Detection of plasma Dz13. An *in vitro* hybridization method was developed for Dz13 and validated by measuring Dz13 in human plasma at various dilutions and at various time points by Charles River Laboratories (Edinburgh, UK). The hybridization assay used a streptavidin-biotin and anti-digoxigenin-POD-based detection system, with 5'AGCTAGCTTTCCTCCCG-Biotin-TEG-3' and 5'digoxigenin-ACAACGCCTCTCGTTG-3' (HPLC-purified, Sigma) to capture and

detect Dz13. Samples of blank plasma were tested to find the limit of detection (LOD), and the lower limit of quantification (LLOQ) was determined from analyzing the standard curve and spiked samples. The LLOQ was determined to be 1·0ng Dz13/ml in whole plasma.

Histological and immunohistochemical analyses

Histology. 5μm thick paraffin sections from each biopsy and excision samples were stained for hematoxylin and eosin (H&E) using the Shandon Varistain 24-4 (Thermo Fisher Scientific Inc, Waltham, MA, USA) and assessed histologically for diagnosis by RPAH pathologists. The tumor depth was measured in millimeters as the vertical distance from the granular layer of the epidermis to the deepest tumor nests [34]. The measurement was performed on the 2 mm punch biopsy samples, which were taken from the center of the target BCC where the tumors were most bulky clinically, and then on the excision samples at the site adjacent to the previous biopsy site. Where it was not possible to determine the biopsy site in the excision sample, the deepest tumor depth was taken for comparison against biopsy sample depth. The tumor mitotic rate was also assessed using the American Joint Committee on Cancer (AJCC) guideline as per standard protocol [35].

Immunohistochemistry. Paraffin sections were prepared for a semi-quantitative assessment of a range of proliferative, proteolytic and apoptotic markers. The clones of the antibodies were recommended for formalin-fixed paraffin-embedded tissue immunostaining by the manufacturers. c-Jun, VEGF-A, FGF-2, MMP-2, MMP-9, Caspase-3, -8 and -9 were stained using the DAKO Autostainer Universal Staining System (DAKO Corporation, Carpinteria, CA, USA), while p53, Ki-67, Bcl-2, CD1A, CD3, CD4, CD8, CD20 and CD68 were stained using the Leica BOND-MAX autostainer (Leica Microsystems, North Ryde, NSW, Australia). All markers were stained using the alkaline phosphatase system according to the standard laboratory protocol outlined in **Supplementary Table 11A** and **11B.** Details of the primary antibodies are listed in **Supplementary Table 12.**

Data analysis. Immunohistochemical results went through a data verification procedure with two forms of analysis by two independent and blinded observers. In the first analysis reported here,

BCC tumor nests were identified manually and positive-staining cells (red chromogen) were quantified in three high magnification fields (400x), and expressed as a percentage of the total number of tumor nest cells in the given fields. In the second analysis, independent automated quantification of the positive-staining area was performed using the DAKO image analysis system (DAKO Corporation) and expressed as a percentage of the total nest area. Digital pictures of each sample were taken using the DAKO AICS Image Scanner. Using the images, a colour threshold was manually calibrated to establish a positive colour threshold for each stain, which was then applied across all sections. The area of interest was selected with a digital drawing tool in the program and the positive staining area was expressed as percentage of the total area selected, in arbitrary units. The entire usable area of the specimen was analysed. Results from the two methododologies (*i.e.* manual count of cell staining positive vs image analysis for assessment of area stained) were formally compared (**Supplementary Tables 6** and **7**) using the Cochran-Mantel-Haenszel test for probability of concordance. For clarity mainly the results from the first method are reported here.

Statistical analyses

All data were recorded in triplicate and monitored by an independent contract research organization, Datapharm Australia Pty Ltd (Drummoyne, NSW, Australia) for accuracy and validity. Data were entered into Symetric Version 9·1·12 (JumpMind Inc, Columbus, OH, USA) and analyzed by the Statistical Operations and Programming Department at Datapharm using SAS version 9.2 (Statistical Analysis System, Cary, NC, USA). Graphs were depicted either as individual values or box plots with median and interquartile ranges. Results were expressed as a mean with standard error of mean for demographic features, and as median with interquartile ranges and 95% confidence intervals for the rest. Significance testing was performed using Wilcoxon signed rank test where applicable and considered significant at p=0·05.

Role of the funding source

The sponsors had no role in the design, data collection, data analysis, data interpretation, and writing of the manuscript.

Supplementary Table 1. Selection criteria for subject recruitment

Inclusion Criteria
1. Patients of either gender aged ≥ 18 years 2. Histologically proven, previously untreated nodular BCC 3. Measurable disease of 5–16mm diameter, measureable in two dimensions, located on trunk or limbs 4. Patients willing and able to participate in all aspects of the study, including completion of subjective evaluations, attending scheduled clinic visits and compliance with protocol requirements as evidenced by providing written informed consent
Exclusion Criteria
1. Nodular BCC lesions located on face 2. Signs of recent sunburn to any site 3. Prior or co-existent malignancies (other than stage I internal malignancy where treated and disease-free for ≥ 5 years, non-melanomatous skin cancer or *in situ* cancer of the cervix) 4. Radiotherapy to > 30% of marrow-bearing bone within the previous three months 5. Known genetic predisposition to skin cancer 6. Clinically significant non-malignant disease 7. Major surgery within the past four weeks 8. Women of childbearing potential. The effects of Dz13/DOPE/DOTAP on the developing human fetus are unknown. For this reason men with women partners of child-bearing potential had to agree to use proven methods of contraception (hormonal or barrier method of birth control; abstinence) for the duration of study participation and 90 days following administration of Dz13 9. Patients currently taking anticoagulant medications, excluding the use of aspirin (≤ 150mg daily) for cardiovascular prophylaxis 10. Current use of immunosuppressant medication 11. Patients with history of liver disease or evidence of significant impairment of liver function (values ≥ 3 times upper limit of normal for AST or ALT), or in the investigator's opinion, liver function impairment to the extent that the subject should not participate in the study 12. Cardiovascular disease ≥ grade 1 as defined by the NCI common Terminology Criteria for Adverse Events (CTCAE v4.0) 13. Evidence of a QT prolongation on initial screening ECGs (QTc ≥ 480 milliseconds) or history of long QT syndrome (or an immediate family member with the condition), or who have hypokalemia or receiving Class 1a antiarrhythmic medications (eg. Quinidine, mexiletine, procainamide) or Class III antiarrhythmic medications (eg. sotalol, amiodarone) 14. Evidence of clinically significant (or with CTCAE v4.0 ≥ 2 grade) respiratory, renal, gastrointestinal, neurological or psychiatric disease, as determined by medical history, clinical laboratory tests and physical examination, that would place the subject at risk upon exposure to the study treatment or that may confound the analysis and/or interpretation of the study results 15. History of immune-mediated thrombocytopenia, thrombotic thrombocytopenic purpura or

other platelet disease
16. Known to be human immunodeficiency virus (HIV), hepatitis B surface antigen or hepatitis C positive; or with a history of chronic active hepatitis or cirrhosis
17. Uncontrolled or serious infection within the past four weeks
18. Patients likely to receive any additional treatment which may interfere with study design or interfere with involvement in the study
19. History of drug abuse (alcohol, cocaine, marijuana or opiates); it was recommended that alcohol consumption be restricted to a moderate level during the study
20. Current enrolment in a clinical study involving any other investigational agent
21. Previous participation in the current study

Supplementary Table 2. Study drug formulation

Dz13 Dose Group (µg)	DMEM (µl)[1]	Dz13 (µl)[2]	DOPE / DOTAP (µl)[3]	Dz13 Dose (µg) per 50µl Formulation
10	82·5	**2·5**	15	10
30	77·5	**7·5**	15	30
100	60	**25**	15	100

[1]DMEM = Dulbecco's modified essential medium; [2]volume of 8mg/ml Dz13 stock solution; [3]volume of mixed DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine) and DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) stock solution containing 10mg/ml of each lipid.

DMEM was used in the Dz13/DOTAP/DOTAP formulation for absolute consistency with that used in the preclinical toxicological studies (reported in Cai *et al.* [1]), all of the Dz13 efficacy studies in BCC- and SCC-bearing mice and the *in vitro* cell culture experiments [1].

Supplementary Table 3. Summary of adverse events by dose cohort and overall

		Number of subjects (%)			
		10 µg n=3	**30 µg n=3**	**100 µg n=3**	**Overall n=9**
Number of subjects reporting at least one treatment emergent AE	(n (%))	2 (66·7)	1 (33·3)	1 (33·3)	4 (44·4)
Number of subjects reporting at least one:					
mild AE	(n (%))	1 (33·3)	1 (33·3)	0 (0·0)	2 (22·2)
moderate AE	(n (%))	1 (33·3)	0 (0·0)	1 (33·3)	2 (22·2)
AE related to study treatment	(n (%))	1 (33·3)	0 (0·0)	0 (0·0)	1 (11·1)
serious AE	(n (%))	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
AE resulting in death	(n (%))	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
AE resulting in study withdrawal	(n (%))	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
Number of treatment emergent AEs reported	(n)	5	1	1	7
mild AEs	(n)	4	1	0	5
moderate AEs	(n)	1	0	1	2
treatment-related AEs	(n)	3	0	0	3
Number of events by action taken:					
no action	(n)	4	1	0	5
remedial therapy	(n)	1	0	1	2
permanent discontinuation of study treatment	(n)	0	0	0	0
other action	(n)	0	0	0	0
Number of events by outcome:					
recovered	(n)	4	1	1	6
continuing at end of study	(n)	1	0	0	1
changed severity	(n)	0	0	0	0

Treatment-emergent adverse events by severity, relationship to study product, MedDRA System Organ Class and Preferred Term

		Mild		Moderate		Severe	
Dose cohort	**System Organ Class Preferred Term**	**Unrelated n (%)**	**Related n (%)**	**Unrelated n (%)**	**Related n (%)**	**Unrelated n (%)**	**Related n (%)**
Overall	Any	2 (22·2)	1 (11·1)	2 (22·2)	0 (0·0)	0 (0·0)	0 (0·0)
	Eye disorders	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Vision blurred	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Gastrointestinal disorders	0 (0·0)	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Nausea	0 (0·0)	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	General disorders and administration site conditions	0 (0·0)	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Injection site discomfort	0 (0·0)	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Injection site swelling	0 (0·0)	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Infections and infestations	0 (0·0)	0 (0·0)	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)
	Wound infection	0 (0·0)	0 (0·0)	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)
	Skin and subcutaneous tissue disorders	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Erythema	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Vascular disorders	0 (0·0)	0 (0·0)	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)

		Mild		Moderate		Severe	
	Phlebitis	0 (0·0)	0 (0·0)	1 (11·1)	0 (0·0)	0 (0·0)	0 (0·0)
10 µg (n=3)	Any	1 (33·3)	1 (33·3)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)
	Eye disorders	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Vision blurred	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Gastrointestinal disorders	0 (0·0)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Nausea	0 (0·0)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	General disorders and administration site conditions	0 (0·0)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Injection site discomfort	0 (0·0)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Injection site swelling	0 (0·0)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Infections and infestations	0 (0·0)	0 (0·0)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)
	Wound infection	0 (0·0)	0 (0·0)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)
30 µg (n=3)	Any	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Skin and subcutaneous tissue disorders	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
	Erythema	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)	0 (0·0)
100 µg	Any	0 (0·0)	0 (0·0)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)

	Mild		Moderate		Severe	
(n=3)						
Vascular disorders	0 (0·0)	0 (0·0)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)
Phlebitis	0 (0·0)	0 (0·0)	1 (33·3)	0 (0·0)	0 (0·0)	0 (0·0)

Supplementary Table 4. **Plasma concentrations of Dz13**

Time	Patient ID								
	0101	**0102**	**0103**	**0201**	**0202**	**0203**	**0301**	**0302**	**0303**
Day 1 0	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1·14	<LLOQ
Day 1 0.5 h	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1·75	<LLOQ
Day 1 1 h	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1·86	<LLOQ
Day 1 2 h	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1·04	<LLOQ
Day 1 4 h	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Day 1 8 h	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Day 1 12 h	<LLOQ	<LLOQ	<LLOQ	1·28	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Day 1 24 h	<LLOQ	<LLOQ	<LLOQ	1·01	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Day 7	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1·15	<LLOQ	1·87	<LLOQ
Day 14	<LLOQ	<LLOQ	1·63	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
Day 28	<LLOQ	<LLOQ	1·36	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1·02	<LLOQ

Results are expressed in ng/ml
LLOQ, Lower limit of quantification was 1·0 ng/ml in whole plasma

Supplementary Table 5. Comparison of biopsy with matched excision samples in nests of BCC without treatment between biopsy and excision

Marker	% positive cells in BCC nests[1]			
	Biopsy[2]	Excision[3]	% Difference[4]	p-value[5]
c-Jun	57·0 (51.0, 61.3) [47.2, 66.0]	65.7 (54.3, 67.7) [50.5, 69.4]	6.1 (-6.9, 16.1) [-6.2, -15.3]	0·4961
Caspase-9	5·0 (3.3, 8.3) [3.7, 7.6]	4.3 (3.0, 5.7) [2.7, 5.3]	-52.9 (-79.6, 0.0) [-145.0, 17.4]	0.0547
CD3	3.7 (2.7, 4.3) [1.6, 6.6]	3.7 (3.3, 4.7) [2.2, 5.0]	0.0 (-54.5, 35.1) [-84.5, 35.0]	0.5781

[1]Percentage cells positive in tumor nests for the specific immunomarkers indicated; [2]biopsy sample expressed as median with (interquartile range, IQR) and [95% confidence intervals]; [3]excision sample removed 11-34 (average 19) days following biopsy expressed as median with (interquartile range, IQR) and [95% confidence intervals]; [4]percentage difference between biopsy and excision samples expressed as median with (interquartile range, IQR) and [95% confidence intervals]; [5]statistical comparison between biopsy and excision samples performed using Wilcoxon signed rank test.

Supplementary Table 6. Dz13 alters expression of c-Jun and proteins involved in tumorigenesis in BCC nests by image analysis assessment of positive area stained

Protein Markers	Positive area by image analysis (arbitrary units)[1]				CMH[6]
	Pre-Dz13[2]	Post-Dz13[3]	Difference[4]	p-value[5]	
c-Jun	9.8 (4.9, 16.3) [6.0, 15.1]	1.8 (1.1, 6.0) [0.9, 6.2]	-5·3 (-9·9, -2·8) [-10.9, -3.2]	0·0039*	#
Caspase-9	0.04 (0.01, 0.09 [-0.5, 1.5]	0.19 (0.01, 1.28) [-0.4, 2.3]	0·14 (0·04, 1·1) [0.0, 1.0]	0·0039*	#
p53	7.3 (2.8, 40.1) [3.8, 40.8]	22.6 (6.5, 56.4) [10.5, 50.8]	5·8 (0·9, 15·7) [2.5, 14.2]	0·0117*	#
MMP-9	0.41 (0.1, 2.5) [-0.5, 3.8]	0.16 (0.1, 0.6) [0.0, 0.9]	-0·12 (-1·8, -0·02) [-3.5, 1.0]	0·0742	#
Bcl-2	28.9 (20.7-52.4) [22.9, 50.0]	38.57 (17.6, 51.8) [22.8, 50.3]	-4·8 (-10.9, 14·2) [-14.5, 14.8]	0·7344	0.0833
Caspase-3	0.01 (0, 0.08) [0.0, 0.2]	0.10 (0.04, 0.44) [0.1, 0.4]	0·05 (-0·03, 0·4) [0.0, 0.4]	0·2031	0.1573
Caspase-8	0.2 (0.1, 2.3) [-0.1, 2.2]	0.6 (0.1, 1.5) [0.0, 1.8]	0·05 (-1·0, 0·7) [-1.6, 1.4]	1·0000	0.0455
Ki-67	14.3 (2.7, 24.5) [5.5, 22.6]	17.5 (8.8, 28.7) [9.9, 26.1]	4·5 (-2·3, 7·6) [-0.9, 8.8]	0·0977	0.0455
MMP-2	0.1 (0, 0.8) [-0.1, 0.9]	0.1 (0, 0.2) [-0.2, 1.0]	-0·05 (-0·7, 0·1) [-0.9, 0.8]	0·6523	0.0833
FGF-2	1.2 (0.5, 6.8) [-0.9, 10.4]	1.3 (0.7, 7.2) [-0.4, 8.8]	0·4 (-4·1, 2·3) [-3.7, 2.6]	0·9102	0.3173

VEGF-A	4.0 (1.5, 13.9) [0.4, 16.8]	8.0 (3.1, 11.8) [4.1, 12.4]	0·1 (-7·1, 7·4) (-8.6, 7.9]	1·0000	0.1573

[1]Positive area in tumor nests for the specific immunomarker indicated as measured by image analysis in arbitrary units; [2]pre-dose biopsy sample expressed as median with (interquartile range, IQR) and [95% confidence intervals]; [3]14 day post-dose excision sample expressed as median with (interquartile range, IQR) and [95% confidence intervals]; [4]absolute difference between pre- and post-dose expressed as median with (interquartile range, IQR) and [95% confidence intervals], could not be expressed as % difference as some samples were zero; [5]statistical comparison between pre and post Dz13 treatment samples performed using Wilcoxon signed rank test; (*) denotes statistically significant p-values; [6]CMH, Cochran-Mantel-Haenszel test for probability of concordance between analysis by % positive cells (data in table 2 in main text) and positive area by image analysis data shown in this table, # indicates test probability could not be calculated as there were no discordant results.

Supplementary Table 7. Dz13 increases lymphocyte and inflammatory cell infiltration in BCC by image analysis assessment of positive area stained

Cell Types	Positive area by image analysis (arbitrary units)[1]				CMH[6]
	Pre-Dz13[2]	Post-Dz13[3]	Difference[4]	p-value[5]	
T Lymphocytes (CD3$^+$)	0.04 (0.0, 0.2) [0.0, 0.2]	0.42 (0.1, 1.3) [0.2, 1.1]	0.42 (0.0, 1.1) [0.1, 0.9]	0.0078*	0.3173
(CD4$^+$)	0.07 (0.0, 0.2) [0.0, 0.2]	0.57 (0.2, 0.9) [0.1, 1.4]	0.56 (0.1, 0.8) [0.0, 1.3]	0.0156*	0.1573
(CD8$^+$)	0.04 (0.02, 0.10) [0.0, 0.1]	0.17 (0.11, 0.94) [0.0, 0.8]	0.11 (0.0, 0.9) [-0.1, 0.8]	0.0391*	0.3173
Dendritic Cells (CD1a$^+$)	0.25 (0.11, 0.41) [-0.3, 1.5]	1.22 (0.36, 3.46) [0.2, 3.7]	0.9 (0.2, 2.0) [0.1, 2.6]	0.0195*	0.1573
B Lymphocytes (CD20$^+$)	0.1 (0.0, 0.5) [0.0, 0.6]	0.1 (0.0, 0.6) [0.0, 0.5]	0.0 (-0.4, 0.4) [-0.5, 0.4]	0.9102	0.0833
Macrophage (CD68$^+$)	0.5 (0.3, 0.8) [0.2, 0.9]	0.6 (0.1, 3.7) [0.0, 3.2]	0.07 (-0.5, 3.4) [-0.7, 2.7]	0.6523	0.3173

[1]Positive area in tumor nests for the specific immunomarker indicated as measured by image analysis in arbitrary units; [2]pre-dose biopsy sample expressed as median with (interquartile range, IQR) and [95% confidence intervals]; [3]14 day post-dose excision sample expressed as median with (interquartile range, IQR) and [95% confidence intervals]; [4]absolute difference between pre- and post-dose expressed as median with (interquartile range, IQR) and [95% confidence intervals], could not be expressed as % difference as some samples were zero; [5]statistical comparison between pre and post Dz13 treatment samples performed using Wilcoxon signed rank test; (*) denotes statistically significant p-values; [6]CMH, Cochran-Mantel-Haenszel test for probability of concordance between analysis by % positive cells (data in table 3 in main text) and positive area by image analysis data shown in this table, # indicates test probability could not be calculated as there were no discordant results.

Supplementary Table 8. Study outline

	Screening	Injection								Assessment 1	Assessment 2	Completion
Day	**-14**	**1**								**7**	**14**	**28**
Time (h)		**0**	**0·5**	**1**	**2**	**4**	**8**	**12**	**24**			
Medical history	X	X										
Physical examination	X	X								X	X	X
Vital signs	X	X		X	X	X	X	X	X	X	X	X
12-lead ECG	X	X		X				X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X
Hematology	X	X		X	X	X	X	X	X	X	X	X
Biochemistry	X	X		X	X	X	X	X	X	X	X	X
Coagulation studies	X	X		X	X	X	X	X	X	X	X	X
Immunology	X	X							X	X	X	X
Pharmacokinetic analysis		X	X	X	X	X	X	X	X	X	X	X
Urinalysis	X	X							X	X	X	X
Tumor micrometry	X	X								X	X	
Photography	X	X								X	X	X
Dermoscopy	X	X								X	X	X
Lesion Biopsy	X											

Lesion excision											X	
Dz13 injection		X										

Supplementary Table 9. Laboratory reference ranges

Tests	Normal Range	Test	Normal Range
Albumin	38-48 g/l	Hemoglobin (male)	130-170 g/l
ALP	30-130 U/l	Hemoglobin (female)	120-150 g/l
ALT	5-55 U/l	Red cells (male)	4·50-5·50 x10^{12}/l
AST	5-55 U/l	Red cells (female)	3·80-4·80 x10^{12}/l
Bicarbonate	24-32 mmol/l	Hematocrit (male)	0·40-0·50
Bilirubin Total	0-21 mol/l	Hematocrit (female)	0·36-0·46
Calcium Total	2·10-2·60 mmol/l	MCV	80-100 fl
Chloride	97-109 mmol/l	MCH	27-32 pg
Cholesterol Total	≤ 5·2 mmol/l	Platelets	150-400 x10^{9}/l
Creatinine (male)	70-110 mg/dl	White cells	4-10·0 x10^{9}/l
Creatinine (female)	50-90 mg/dl	Neutrophils	2-7·0 x10^{9}/l
GGT (male)	< 60 U/l	Lymphocytes	1·0-3·0 x10^{9}/l
GGT (female)	< 35 U/l	Monocytes	0·2-1·0 x10^{9}/l
Glucose Random	3·0-7·7 mmol/l	Eosinophils	0-0·5 x10^{9}/l
Magnesium	0·7-0·95 mmol/l	Basophils	0-0·1 x10^{9}/l
Phosphate	0·8-1·5 mmol/l	INR	0·9-1·2
Potassium	3·5-5 mmol/l	PT	12-15 sec
Protein Total	62-80 g/l	APTT	25-37 sec
Sodium	135-145 mmol/l	IgG	6·39-15.6 g/l
Triglycerides (male)	< 2.5 mmol/l	IgA	0·7-3·12 g/l
Triglycerides (female)	< 2 mmol/l	IgM	0.5-3 g/l
Urea	3-8 mmol/l	IgE	0-200 kU/l

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; INR = international normalized ratio; PT = prothrombin time; APTT = activated partial thromboplastin time; IgG, A, M and E = immunoglobulins G, A, M and E

Supplementary Table 10. Dose limiting toxicity

DLT was assessed over 14 days following study drug administration. All toxicities or adverse events were graded according to the Common Toxicity Criteria for Adverse Events (CTCAE) Version 4.0. A dose limiting toxicity (DLT) is toxicity or an adverse event which meets one of the following criteria:

I. Any Grade 3, non-haematologic toxicity except:
• nausea and vomiting (NB any ≥ grade 3 nausea or vomiting which is not responding to medical management will be considered a DLT)
• fever (in the absence of neutropenia)
• asymptomatic hyperglycaemia
• asymptomatic hyperuricaemia

II. Any Grade 4 toxicity:
• except asymptomatic hyperuricaemia

III. Haematological toxicities:
• Febrile neutropenia (ANC < 1 x 10^9/L and fever > 38·5˚C)
• Grade 4 neutropenia (ANC < 0·5 x 10^9/L) for seven or more days

Supplementary Table 11A. Alkaline phosphatase immunohistochemical staining protocol (using DAKO Autostainer and EnVision G|2 System/AP, cat K5355)

	Step	Reagent	Time
1	Deparaffinise	Xylene	10 min x 2
2	Rehydrate	Graded ethanol	2 min each
3	Antigen Retrieval	pH 9·0 EDTA or pH 6·0 Citrate Buffer (DAKO Corporation, Carpinteria, CA, USA) at 100^{o}C	45 min
4	Endogenous AP Block	DAKO Dual Endogenous Enzyme Block	30 min
5	Protein Block	DAKO Serum-Free Protein Block	30 min
6	Primary Antibody	*See* **Supplementary Table 12**	60 min
7	Secondary Reagent	Rabbit/Mouse LINK (DAKO)	30 min
8	Tertiary Reagent	AP Enzyme Enhancer (DAKO)	30 min
9	Substrate	Permanent Red Chromogen (DAKO)	15 min
10	Counterstain	Hematoxylin	5 sec
11	Air-dry & Mount	Tissue-Tek Tissue-Mount (Sakura Finetek, Alphen aan den Rijn, Netherlands)	

Supplementary Table 11B. Alkaline phosphatase immunohistochemical staining protocol (using Leica BOND MAX Autostainer and Bond Polymer Refine Red Detection system, cat DS9390)

	Step	Reagent	Time
1	Deparaffinise	Xylene	10 min x 2
2	Rehydrate	Graded ethanol	2 min each
3	Antigen Retrieval	Novocastra Bond Epitope Retrieval Solution 1 (ER1-pH 6·0) or Solution 2 (ER2-pH 9·0) at 100°C (Leica Microsystems, North Ryde, NSW, Australia)	20-30 min
4	Primary Antibody	*See* **Supplementary Table 12**	30 min
5	Secondary Reagent	Post-primary AP (Leica)	20 min
6	Tertiary Reagent	Polymer AP (Leica)	30 min
7	Substrate	Fast Red (Leica)	20 min
8	Counterstain	Hematoxylin (Leica)	5 min
9	Air-dry & Mount	Surgipath MM24 (Leica)	

Supplementary Table 12. Primary antibodies used for immunohistochemistry

Marker	Dilution	Retrieval	Company
DAKO Autostainer			
c-Jun	1:100	EDTA	Abcam (Cambridge, UK)
VEGF-A	1:400	EDTA	Abcam
FGF-2	1:100	EDTA	Santa Cruz (Santa Cruz, CA, USA)
MMP-9	1:1000	EDTA	Abcam
MMP-2	1:100	Citrate	Abcam
Caspase-3	1:10	Citrate	Abcam
Caspase-8	1:100	Citrate	Santa Cruz
Caspase-9	1:100	Citrate	Santa Cruz
Leica BOND MAX			
p53	1:800	ER2	Invitrogen (Invitrogen Australia, Mulgrave, VIC, Australia)
Ki-67	1:100	ER2	Novocastra (Leica Microsystems, North Ryde, NSW, Australia)
Bcl-2	1:200	ER2	Novocastra
CD1A	1:50	ER2	Novocastra
CD3	1:200	ER2	Novocastra
CD20	1:100	ER2	Novocastra
CD68	1:100	ER2	Novocastra
CD4	1:100	ER2	Novocastra
CD8	1:100	ER2	DAKO